HUMAN IMMUNOFLUORESCENT VIRUS ANTIBODIES AND ANTIGEN IN INFANTS BORN TO SEROPOSITIVE MOTHERS

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Human Immunodeficiency Virus Antibodies and Antigen in Infants Born to Seropositive Mothers

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TABLE OF CONTENTS

ABSTRACT

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The diagnosis of HIV infection in infants born to infected mothers is difficult. Because of transplacental acquisition of maternal antibody all infants born to seropositive mothers are Ab-positive at birth. In order to identify serologic features which distinguish those infants who are truly infected twenty-five infants born to HIV seropositive mothers were followed prospectively. At 10-25 months of follow-up, 3 had AIDS, 8 had signs such as growth retardation and developmental delay consistent with but not specific for HIV infection, and 14 were healthy. Using Western immunoblotting, serial serum samples from 22 of these infants and 6 children identified after the onset of clinically evident HIV infection were tested for antibody to two different strains of HIV. Examination of the serial antibody patterns revealed that appearance of new antibody, disappearance of antibody, and maintenance of antibody present at birth are all associated with HIV infection. Of 16 infants who lost antibody completely, 7 either had AIDS or were thriving poorly. These 7 infants lost their antibody at an earlier age than those who were asymptomatic suggesting that rapid loss of antibody may be indicative of active infection. Densitometric analysis of immunoblots suggests that the half-life of passively acquired antibody is 38 ± 3 days. HIV core antigen (p24) was detected in 2 of 20 infants identified prospectively, 3 of 10 children identified after onset of clinically evident HIV infection, and 4 of 18 mothers of children in either group. Four of the antigen-positive children had AIDS; the fifth had signs consistent with HIV infection. None of the asymptomatic children were antigen positive. Each of the antigen-positive mothers had a child with AIDS. These findings suggest that antigen detection is specific but not sensitive for symptomatic HIV infection, and that presence of antigen in a mother's serum may be an early marker for HIV infection in her child.

INTRODUCTION

Since 1981 46,000 cases of Acquired Immunodeficiency Syndrome (AIDS) have been reported in the United States[1]. Of these 1.4%, or 640, have been children less than 13 years old[2]. Some of the pediatric cases have been attributable to transfusion of infected blood products. By far the majority, however, have occurred as a result of vertical transmission from mothers who themselves have AIDS risk factors[3].

The etiologic agent of AIDS, previously known as human T-cell lymphotropic virus type III (HTLV-III), lymphadenopathy-associated virus (LAV), or AIDS-related virus (ARV), and now designated human immunodeficiency virus (HIV), was identified in 1983-84[4-6]. Characterization of this retrovirus at the molecular and biochemical levels has been rapid. The entire genome has been sequenced[7-10]; eight genes and several gene products have been identified[11-16]. Progress in understanding HIV transmission, however, especially its vertical transmission, has been slow. Many questions remain unanswered. For example, the rate of transmission from infected mother to offspring is not known, and the route of this transmission have not been determined. Such information is important for several reasons. Most obviously it will enable more effective counseling of pregnant women and might make possible interventions to prevent future cases of pediatric AIDS. In addition such information may contribute to the understanding of the pathogenesis of HIV infection in general. Much of what is known about the infectivity of other viruses, particularly hepatitis B virus, came from studies of maternal-fetal transmission [17,18]. Because children have been exposed to and

harbor fewer infectious agents than adults possible cofactors important to the development of AIDS may be more readily discernible in children [19].

Difficulty in acquiring information about maternal-fetal transmission of HIV infection has, in part, been due to the problems of diagnosing HIV infection in infants. Because of transplacental transfer of certain immunoglobulins the conventional methods of diagnosis of HIV infection in adults (antibody detection by ELISA or Western blot) are less useful in newborns. The duration of persistence of passively acquired antibody has not been established making interpretation of serologic findings difficult even at several months of age. Other approaches such as detection of IgM specific antibody, detection of viral antigen, and virus isolation have not yet been shown to be sensitive methods for diagnosing HIV infection in infants.

This thesis reports results of HIV antibody and antigen testing in a group of prospectively identified infants born to HIV infected mothers. The goal was to identify serologic features which distinguish infected infants. The following questions were addressed:

— Does the disappearance (or appearance) of HIV antibody correlate with clinical outcome?

— Does the loss of antibody to particular viral polypeptides correlate with clinical outcome?

— What is the half-life of maternal antibody?

— Is the presence of HIV antigen in a child a useful predictor of forthcoming disease?

— Is the presence of HIV antigen in the mother a useful predictor of infection in her child?

REVIEW OF THE LITERATURE

I. Pediatric AIDS: Epidemiologic, Clinical, and Immunologic Features

In late 1983 and early 1984 four papers, one each from New York, Newark, Miami, and Toronto, described infants and children with a new immunodeficiency syndrome[20-23]. The clinical and immunologic features similar to those of adult AIDS, included failure to thrive, hepatosplenomegaly, lymphadenopathy, recurrent infections, reversed T4/T8 lymphocyte ratio, and depressed cell-mediated immunity. The common risk factors among these children appeared to be parental intravenous (IV) drug abuse, sexual promiscuity, and Haitian background. Only a few had received blood transfusions. These cases, described prior to the identification of the human immunodeficiency virus, were felt to support an infectious etiology of AIDS. Subsequent to these reports a very general definition of pediatric AIDS was established by the CDC[24].

The CDC case definition of pediatric AIDS has been modified twice as more data has been gathered and as serologic testing has been developed[25,26]. Current criteria for diagnosis of full-blown AIDS include positive HIV culture or detectable HIV antigen, or positive serology plus either an opportunistic infection, multiple serious bacterial infections, or histologically-confirmed lymphocytic interstitial pneumonitis (LIP). To date 640 cases of pediatric AIDS have been reported. 19% were either hemophiliacs or recipients of blood transfusions; almost all of the remainder had at least one parent with a risk factor for AIDS, most commonly IV drug abuse[3]. Some of the mothers of these children had AIDS or AIDS related complex (ARC); some were seropositive but asymptomatic. Several mothers,

asymptomatic at the time their child presented with AIDS, developed AIDS years later [27]. It has become evident that infected mothers can give birth to more than one affected child, or they can give birth to both affected and unaffected children [28]. Little evidence exists to support horizontal spread of HIV from infected children. Several investigators following household contacts and close caregivers of infected infants and children have found no seroconvertors[19,29]; there is only one case report of such an occurrence[30].

The clinical features of pediatric AIDS have been well described[28,31]. Almost all children have lymphadenopathy, hepatosplenomegaly, and failure to thrive. Parotid gland enlargement, seen in several early cases, actually occurs in less than 10% of affected children[31]. Recurrent diarrhea, (often without an obvious infectious etiology), retarded psychomotor development, or other neurologic abnormalities occur in 10-50% [31,32]. A set of craniofacial dysmorphisms including microcephaly, prominent, boxlike forehead, ocular hypertelorism and flattened nasal bridge have been described in a group of children with AIDS [33,34]. This is not, however, a universal feature.

Children with AIDS are extremely susceptible to bacterial infections, more so than their adult counterparts. The infections, typically resulting from common organisms such as *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae,* and *Salmonella,* include otitis media, pneumonia, cellulitis, and sepsis. One study of 60 children with AIDS found that 40% had at least one episode of sepsis; in many cases this was the presenting feature[28]. Opportunistic infections are also seen in pediatric AIDS. The most common include *Pneumocystis carinii* pneumonia (PCP), disseminated *Mycobacterium avium*

intracellulare (MAI) infection, disseminated cytomegalovirus (CMV), and mucocutaneous Candida[35]. Kaposi sarcoma, present in 20-33% of adults with AIDS, occurs rarely in the pediatric population[36].

Lung disease in pediatric AIDS is not confined to the above-mentioned organisms. Many children with AIDS - as many as 70% by one estimate[31] - develop chronic lymphocytic interstitial pneumonitis (LIP) without a documenented etiologic agent. Chest roentenogram shows a nodular infiltrate; histologic exam reveals diffuse infiltration of alveolar septa and peribronchiolar areas by lymphocytes and plasma cells. The demonstration of Epstein Barr virus (EBV) nucleic acid in lung biopsy tissue and/or the marked elevation of antibody titer to the EBV viral capsid and early antigens in some of these patients suggest that LIP may represent a lymphoproliferative response to EBV infection[37]. Interestingly, LIP is extremely rare in adults with AIDS.

The natural history of childhood AIDS has not been well defined. The diagnosis of AIDS is usually made after 3 months of age. The age of presentation in three studies ranged from 1 month to 6 years, with a mean of 5 months in one study[38], 6 months in another study[29], and a median of 18 months in yet a third study[39]. There is some suggestion that children are more susceptible than adults to the pathogenic effects of HIV. The incidence of transfusion associated AIDS in infants is six-fold that of adults[40], and, as of 1986, 65% of the children with AIDS had died as compared to 50% of the adults with AIDS suggesting a more rapid course of disease[31]. However, in contrast to those children who develop severe opportunistic infection and die before two years of age, there is a group of children, often those with LIP, whose disease has a much more stable, indolent course[39].

Like adults, most infants and children with AIDS have impaired cell-mediated immunity, and many (80%) have a T4/T8 ratio less than 1[11]. However, in contrast to adults, they are not usually lymphopenic: the reversed T cell subset ratio is due to an increase in number of T8 cells as opposed to a decrease in number of T4 cells[28]. It has been postulated that the increase in T8 cell number is due to concurrent viral infections (such as EBV and CMV) known to increase cytotoxic and suppressor lymphocytes[39].

B cell abnormalities, consisting of high immunoglobulin levels and a poor antibody response to challenge with new antigens, are common in pediatric AIDS. The polyclonal hypergammaglobulinemia, seen in 75% of pediatric AIDS[31], may be a direct effect of HIV infection. Several investigators have shown that polyclonal proliferation of B cells can be achieved *in vitro* by adding HIV to B cells[41-43]. In general, these B cell defects precede the T cell abnormalities and are more pronounced. It has been postulated that this initial selectivity of the B cell deficiency reflects the timing of infection; i.e., the HIV infection may occur at a critical time in the maturation of the B cell system[29].

Other laboratory findings in pediatric AIDS include thrombocytopenia[44], and increased incidence of autoantibody formation. Antinuclear antibodies and antilymphocyte antibodies have been demonstrated in both pediatric and adult AIDS patients[27].

II. Vertical Transmission of HIV: Route and Risk

The most common source of HIV infection for a child is his/her mother. Except for a small number of transfusion-related cases[45-48], which are decreasing

in frequency as a result of effective screening of blood products, children with AIDS have mothers at risk for the disease. The precise route of transmission is not known. Possibilities include transplacental infection, infection during passage through the birth canal, and postnatal infection via breast mild or close contact. Evidence exists for all three. Interruption of transmission from mother to child requires a detailed understanding of the underlying processes and the relative risks associated with each route.

Evidence for intrauterine infection comes primarily from case reports of AIDS in infants born by Caesarian section[49,50], and virus isolation from placenta and fetal tissues[51,52]. In addition, the craniofacial abnormalities observed in some children with AIDS suggests an embryopathic effect of HIV.

The evidence for infection during the birth process (exit infection) is indirect. The presence of IgM antibody to HIV in serum of a single infant at 4 weeks of age but not earlier is one example[53]. If the infection had occurred *in utero*, IgM might be expected to have been present at birth. The age of onset of symptoms of HIV infection also might reflect the time that infection occurred. One explanation for the relatively late onset of disease in children with LIP, and its localization to the lungs, is that in these children the infection was acquired during passage through the birth canal[54]. Isolation of virus from cervical and vaginal secretions of seropositive women also helps support this route of transmission[55,56].

Transmission of the virus after birth has been implicated in two cases of HIV infection in infancy[57,58]. Each child was born to a mother reported to have acquired the infection from a postpartum blood transfusion. Since the mothers breastfed their children, the authors suggested breastfeeding as the possible mode of transmission. HIV has been isolated from the breast milk of infected women[59].

The bulk of the evidence favors intrauterine infection and exit infection. The data, however, is limited. No systematic investigations have been conducted, for example, to determine when during gestation such transmission is most likely to occur. The facial dysmorphisms that have been reported suggest early effects of the virus; however, this embryopathy is by no means universal and does not rule out transmission at later stages of development.

Estimates of the risk of HIV transmission from infected mother to offspring range widely. In one study 20 women were identified as HIV positive because AIDS developed in their previous children. The women subsequently gave birth to 20 infants, 13 of whom (65%) developed AIDS[60]. In two other studies with similar designs 11 of 17 (64%) and 4 of 12 (33%) infants developed AIDS[29,27]. However, because the mothers were identified on the basis of already having had a child with AIDS, the results of these studies cannot be extrapolated to seropositive women in general. Differences in the virulence of virus strains, viral load, or host susceptibility may change the rate of maternal-fetal transmission; studying children born to women known to have already transmitted the virus to offspring may select for these qualities and thereby over-estimate the risk. Other studies found transmission rates to be less than 25%. The conclusions which can be drawn from these studies, are limited by the small number of subjects (three mothers) and short follow-up of the infants (one year) in one study[61], and by observation of symptomatic rather than infected mothers in the other[62].

Preliminary data are available from the only study that is following large numbers of infants born to HIV seropositive mothers[63]. At follow-ups ranging from 1 to 15 months (mean 6 months), 5 of 71 infants had developed AIDS or

ARC. An additional 4 infants had nonspecific symptoms such as failure to thrive and lymphadenopathy. Because diagnosis of childhood AIDS is based heavily on clinical symptoms, longer follow-up is necessary to determine the rate of transmission in this group. The initial data do suggest, however, that women who are symptomatic during pregnancy are more likely to transmit the virus than those who are asymptomatic.

III. HIV Antibody in Infants Born to Seropositive Mothers

Because maternal immunoglobulins cross the placenta, all infants born to HIV seropositive mothers are "positive" by ELISA and/or Western blot whether or not they are actually infected. Therefore, serologic methods employed routinely on adults for diagnosis of HIV infection are not as useful for infants. Only those infants who make antibody against specific HIV polypeptides not recognized by their mother's serum can easily be identified as infected. This situation does not often occur. The earliest reported appearance of new antibody to polypeptides not recognized by maternal serum was at 3 months of age[64]. Prior to that the infant's Western blot pattern was identical to that of his mother. In most cases, infected mothers who complete a pregnancy have antibody to most or all of the HIV polypeptides that can be demonstrated by Western blot. Clearly this decreases the likelihood of identifying reactivities unique to the infant.

Six months is sometimes cited as the age after which antibody in infant sera can be considered endogenous rather than that of the mother[39]. In fact, the length of persistence of maternal antibody to HIV is not known. There is only one published attempt to systematically determine this. The preliminary data from this

prospective study of children born to HIV-positive mothers found that the length of persistence of antibody present at birth (assumed to be maternal) ranged from less than 20 weeks to greater than 11 months. The median age at loss was 10 months. Only 18 of the 71 children being followed had actually become seronegative by the time of the report. By lifetable analysis it was estimated that 25% would still be seropositive at 1 year of age[63].

Unfortunately reversion to a seronegative state may not indicate absence of infection. Because of the immaturity of the immune system, pathogenic effects of HIV on the immune system, or masking of antigen by maternal antibody (as has been proposed for hepatitis B infection[65]), infants may not demonstrate a good antibody response. One study found that in some children with AIDS the presence of antibodies by Western blotting could only be shown after the use of bridging anti-immunoglobulin, and the children, especially the younger ones, recognized fewer polypeptides on Western blot than did their parents[19]. These children were identified after the onset of symptoms; it is not known whether they always had weakly reacting sera or whether they lost antibody as their disease progressed. Loss of antibody, particularly that directed against HIV core protein p24, has been demonstrated in adults just prior to the onset of clinical disease[66-68], and in a small number of infants infected via blood transfusion[69].

The presence of viral specific IgM in newborn sera is the basis for diagnosis of many congenital infections since immunoglobulins of this class cannot cross the placenta. This approach has been attempted for congenital HIV diagnosis but with thus far limited success. The first demonstration of an IgM response to HIV infection was in an animal model for HIV infection[70]. Two of three chimpanzees

transiently produced anti-HIV IgM following infusions of plasma from AIDS patients. IgM has been found in humans as well. In one study, six of 30 adult patients with ARC, and nine of 52 adults at risk for HIV had IgM antibody detected by indirect immunoflurescence[71]; 5 of 65 IgG seronegative adult IV drug users were IgM positive by ELISA in another study[72]. In a third study HIV IgM was detected by radioimmunoassay in nine adult males at risk for infection[73]. The length of the IgM response ranged from 1 week to 38 weeks in these individuals. While these studies suggest a primary and sometimes long-lived IgM response to HIV infection and suggest that, in some instances, IgM antibody is the only marker of HIV infection in adults, the accuracy and sensitivity of the detection methods have not been rigorously investigated. This is particularly crucial with IgM assays because of the frequently encountered nonspecificity of IgM binding[73] and the potential nonspecificity of anti-IgM reagents used in these assays. In only one of the studies[73], for example, was removal of other classes of immunoglobulin included in the procedure.

Attempts to identify IgM in infants have been reported as well. Four of 27 infants born to HIV seropositive mothers had IgM demonstrated by immunoblotting[74]. All four of these infants were greater than three months of age. None of 12 who were younger than 3 months had HIV IgM, and ten children without IgM later developed their own IgG indicating that they were, in fact, infected. Similarly, the infant described previously[63] who, at 3 months, developed IgG against two polypeptides not recognized by his mother's serum, did not have detectable IgM on any of the 3 occasions tested (2, 3, and 4 months of age). Another infant from whom serial serum samples were collected every 4 weeks from birth to 5 months had no detectable IgM until 4 weeks. The quantity of IgM peaked

at 8 weeks and rapidly disappeared thereafter[53]. These studies suggest that the IgM response to HIV infection is weaker and more variable than in other congenital infections and that if present in infants may be very brief.

IV. HIV Antigen Detection

The first HIV antigen assays were developed in 1986[75,76]. Most are similar to the ELISAs used for detecting antibody except that HIV antibody rather than antigen is used as the "capture" agent. These assays were designed initially to detect antigen in tissue culture and their sensitivity and specificity for detecting antigen in human serum has not been established. Nevertheless, data obtained thus far[77-79] suggests that antigen detection may provide an important complement to antibody detection in diagnosing HIV infection and in studying its pathogenesis.

One study found antigen in serum of 86% of 22 adult and pediatric patients with AIDS but in only 8% of seropositive asymptomatic adults. Of 35 patients who seroconverted during the study period antigen was detected in 11[77]. In 5 of 11 antigen was present prior to detection of antibody. Three of eleven individuals remained seropositive for the entire 6-12 months of follow-up; the remaining 8 lost antigen but retained antibody. A second study by the same investigators found that of 19 persons positive for antibody to the HIV envelope protein gp41, 7 lacked antibody to HIV core protein p24. All 7 without antibody to p24 were antigen positive. None of the 12 with core antibody had detectable antigen[80]. The findings of these studies together with the observed association between loss of antibody to p24 and progression of clinical disease[66-69], suggest the following hypotheses: 1) viral antigen circulating in serum may precede antibody production, 2) antigen may

disappear during a latent or asymptomatic phase of the infection, and 3) the presence of antigen after seroconversion indicates worsening of disease. The decrease in antibody to p24 associated with both presence of antigen and progression of disease could be due either to exhaustion of the immune response or to increase in viral replication resulting in antigen excess and complete binding of available antibody.

Antigen detection in congenital HIV infection is limited to 5 pediatric patients with AIDS included in the study described above[75], and a group of 9 infants who had immunologic and clinical evidence of AIDS but lacked antibody by at least one detection method. All were antigen positive[81]. The data suggest that antigen testing should be combined with antibody assays for diagnosis of HIV infection. The studies do not assess the utility of antigen testing in asymptomatic children at risk for HIV infection, and there are no studies thus far which look for a relationship between presence of antigen and infectivity (such as that established for hepatitis B e antigen).

V. AIDS in New Haven

As of December 20, 1986, the incidence of AIDS in the New Haven standard metropolitan statistical area (SMSA) was 142 cases/million, the 12th highest incidence in the country. In the City of New Haven itself the incidence was significantly higher: 634 cases/million. This incidence is almost identical to that of the SMSAs of New York and San Francisco. The AIDS population here is somewhat unique. It is disproportionately represented by women, IV drug users, and blacks. Twenty-nine percent of AIDS patients in New Haven SMSA are

women, compared to a national average of 7 percent. Sixty-one percent of the AIDS patients are black and 43 percent are IV drug users. Not surprisingly, given the large number of infected women, the pediatric AIDS population in New Haven is high. The earliest recognized case was a boy born in 1979[19]. Since 1983, twenty-three other children with clinically-evident HIV infection have been identified[39]. An additional 54 seropositive infants are currently followed in the Yale Pediatric AIDS Clinic.

METHODS

I. Subjects

A. Infants born to HIV seropositive mothers (N = 25)

These infants are part of a prospective study group (PSG) started at Yale-New Haven Hospital (YNHH) in December 1985. HIV-infected mothers were identified with the assistance of New Haven drug dependency units, and the YNHH Women's Center, newborn nursery, Newborn Special Care Unit and pediatric Primary Care Center. As of November 1987 the PSG consisted of 50 infants of such mothers; 25 were included in the current study. Serial serum samples were collected at birth and every 3-6 months thereafter. A total of 72 samples were obtained. Serum was stored at -20°C.

B. Infants and children identified after the onset of clinical signs of HIV infection (N = 10)

These infants and children, designated the Symptomatic Study Group (SSG), presented to YNHH with clinical signs of HIV infection. All were born to seropositive mothers, either IV drug users or sexual partners of IV drug users. A total of 21 serum samples was obtained for this study.

C. HIV-infected mothers (N = 18)

One serum sample was obtained from each of 11 mothers of PSG infants (within 1 month of delivery) and from 7 mothers of SSG children.

II. Clinical Data

Information regarding the clinical status of the infants and children was obtained from the records of the Pediatric AIDS Clinic. Each child was classifed into one of the following groups:

1. Asymptomatic
2. Thriving Poorly
3. AIDS

There currently is no agreed upon definition of pediatric ARC. For this study a child was labelled as "thriving poorly " if he/she did not meet the criteria for AIDS (see below) but had positive serology on at least one occasion plus any of the following:

1. Growth Retardation —weight and height below 5th percentile or movement from one growth curve to a lower one
2. Developmental Delay —assessed by the Denver Developmental Scale
3. Persistent lymphadenopathy, hepatomegaly, splenomegaly or parotitis.
4. Persistent or unexplained recurrent diarrhea
5. More than one bacterial infection requiring hospitalization

The CDC case definition of Pediatric AIDS[26] was used in this study. Any child with positive serology on at least one occasion plus either an opportunistic infection or biopsy-proven lymphocytic interstitial pneumonitis (LIP) was considered to have AIDS.

III. HIV Infected and Uninfected Cells

The HIV-infected cells used as sources of antigen for the Western immunoblots.were H9/RF2 and X50-7.8/PH1-MN. The HIV strains, RF2 and

PH1-MN, were isolated from a Haitain patient with AIDS (RF2) and a child with ARC (PH1-MN). Both strains were provided by Robert Gallo (Bethesda, MD). H9 cells are a subclone derived from the HT T-cell leukemia line[4]. X50-7 cells are human umbilical cord lymphocytes immortalized *in vitro* by EBV. They contain a tightly latent complete EBV genome[82]. The X50-7.8 clone is particularly susceptible to HIV infection. Uninfected H9 and X50-7.8 cells were used as negative controls. Every serum was tested against a panel of antigen from both the infected and uninfected cells in order to distinguish adequately between reactivity to viral proteins and normal cell proteins. All cells were grown in RPMI 1640 plus 10% fetal calf serum with penicillin (50 µg/ml), streptomycin (50 µg/ml) and amphotericin (50 µg/ml).

IV. Immunoblots

Cellular extracts were prepared by harvesting cells and resuspending them in SDS electrophoresis sample buffer (125 mM Tris [pH 6.8], 2% SDS, 10% glycerol, 0.1% 2-mercaptoethanol, 0.2% 35mM phenylmethylsulfonyl fluoride, and bromphenol blue) at a concentration of 10^8 cells/ml. The extracts were sonicated and boiled for 5 minutes just before loading; they were electrophoresed overnight on 20 x 20 cm 12% acrylamide/ bis-acrylamide (30:0.8) gels at 40V. The proteins were then transferred from the gel to nitrocellulose filter paper at 200 mA for 4 hrs. The filter paper was blocked for 1 hour with "Blotto" (5% wt/vol nonfat dry milk, 0.01% antifoam, and 0.002% thimerosal) to prevent nonspecific binding. After blocking, the filters were incubated with serum (previously heated to 60 °C to inactivate virus) diluted 1:100 in Blotto for 1 hr at room temperature

(23°C). After washing (10 mM Tris, 16 mM NaCl, % Tween) the filters were incubated for 1 hr with ^{125}I-labelled staphylococcal protein A (New England Nuclear). After a final wash the nitrocellulose filter was air-dried and exposed to XR O-MAT film (Eastman Kodak, Rochester, NY) for 48 hours at -20°C with intensifying screens. Negative blots were reexposed to film for up to 1 week.

In order to compare relative amounts of antibodies present in different serum samples multiple immunoblots were prepared simultaneously using the same preparations of cell extracts for all blots. The incubations with serum and ^{125}I-staph A were done in parallel with the same reagents.

V. Immunoblots with Bridging Antibodies

Several of the serum samples which were negative for antibody by immunoblot were retested with the use of bridging Abs in an effort to increase signal or to detect IgG subclasses and Ig classes that do not react with staphyloccal protein A. The bridging Ab was rabbit IgG fraction antibody to human light chains (Cal Biochem, La Jolla, CA). The nitrocellulose filter was blocked and incubated with the patient's serum as usual and then for an additional 1.5 hr with the bridging antibody diluted 1:100 in Blotto. The filters were then incubated with iodinated staphyloccal protein A, which also binds well to rabbit IgG.

VI. Determination of Antibody Half-Life

The relative quantities of the HIV antibodies present in serial serum samples were determined by densitometric readings of the immunoblot bands. A Joyce-

Loebl densitometer was used. The log of antibody quantity (i.e., area under densitometer tracing) was plotted against time and antibody half-life was calculated according to the following equation:

$$t_{1/2} = \frac{\log_{10} 2}{\text{slope}}$$

where $t_{1/2}$ equals half-life, and slope equals: $\frac{\Delta \log [\text{antibody quantity}]}{\text{time}}$.

VII. Detection of HIV Antigen

Sera were tested for HIV p24 antigen using two commercially available enzyme-linked immunoassays (Abbott Laboratories and DuPont Laboratories). All samples and controls were assayed in duplicate according to the instructions of the manufacturer. The Abbott assay was performed as follows: 200µl of sample was incubated overnight at room temperature with a bead coated with polyclonal human anti-HIV. Beads were washed with distilled water and incubated for 4 hr at 37°C with rabbit IgG Ab to HIV p24. Beads again were washed and incubated for 2 hr at 37°C with horseradish peroxidase-conjugated goat anti-rabbit IgG. After a final wash the beads were incubated with o-phenylenediamine for 30 minutes at room temperature in the dark. 1N sulfuric acid was added and the A_{492} was read with a spectrophotometric plate reader. The presence or absence of HIV antigen was determined by comparing O.D. of sample with O.D. of HIV Ab negative serum from an individual with no risk factors for HIV infection. A sample was considered positive for antigen if its mean O.D. was greater than the negative control mean O.D. plus 0.050.

The DuPont assay was similar. Two hundred µl of sample was incubated overnight at room temperature with polyclonal rabbit anti-HIV p24 fixed to microtiter plate well. Wells were washed with a PBS/Tris solution and incubated with biotinylated rabbit anti-HIV p24 for 1 hr at 37 °C. The wells again were washed and incubated with streptavidin-horseradish peroxidase for 15 minutes at room temperature. O-phenylenediamine was added following a final wash. The wells were kept at room temperature in the dark for 30 minutes at which time 4N sulfuric acid was added. The A_{492} was read with a spectrophotometric plate-reader. Quantitation of p24 Ag was done by comparing the 0.D. of sample with the O.D.s of HIV viral lysates with known concentrations of p24. A sample was considered positive if its concentration of p24 antigen was at least two times that of HIV Ab negative serum from an individual with no risk factors for HIV infection.

RESULTS

I. Clinical Findings

As of January, 1988 the clinical status of the 25 infants in the prospective study group (PSG) was as follows: 3 had AIDS, 8 were thriving poorly, and 14 were healthy. Two of the infants with AIDS had died, the third has recovered from *P. carinii* pneumonia. The most frequent sign among the children who were thriving poorly was growth retardation, especially poor weight gain; some children manifested signs of developmental delay. Recurrent diarrhea, lymphadenopathy, hepatomegaly, facial dysmorphism, and *Neisseria meningitidis* infection were also observed in this group. The length of follow-up ranged from 10 months to 25 months; the median was 16 months.

Of the 10 children in the symptomatic study group (SSG), i.e., those born to HIV-infected mothers but identified only after AIDS was clincally evident, 4 have died. Three died between 2 and 3 years of age and one died at 6 years.

II. Recognition of HIV Proteins on Western Immunoblot Using Sera from HIV Seropositive Infants

Figure 1 shows an immunoblot of serum from a neonate born to an HIV Ab-positive mother. Seven clusters of HIV polypeptides with the following molecular weights are recognized: 24 kDa, 41 kDa, 46/49/53 kDa (triplet), 60/61 kDa (doublet), 70 kDa, 96 kDa, and 120 kDa. Two other clusters with molecular weights of 17 kDa and 33/35 kDa (doublet) were recognized by other seropositive individuals. Three of these proteins have been characterized. The 24 kDa

polypeptide (p24), encoded by the *gag* gene, is a structural component of the viral core. The 41 kDa glycoprotein (gp41) is a transmembrane protein of the viral envelope and is thought to mediate fusion of susceptible host cells. The major envelope glycoprotein, gp120, binds to the CD4 receptor on the host cell surface, thereby initating infection. In several individuals the 120 kDa glycoprotein was recognized when serum was reacted with X50-7.8/PH1-MN cell extract but not with the H9/RF2 extract. In contrast, the 46/49/53 kDa triplet was in many cases better recognized with the H9/RF2 antigen preparation. In Figure 1 reactions with cell-associated proteins can be seen in both cell lanes containing extracts from uninfected cells (X50-7.8 and H9).

III. Comparison of Antibodies Present in Maternal and Neonatal Serum

Because antibody that is present in infant serum but not in maternal serum would be one indicator of congenital HIV infection, immunoblots of neonatal serum (obtained within one month of birth) from 15 of the PSG infants were compared with immunoblots of corresponding maternal serum. Figure 2 shows a representative infant/mother pair of blots. None of the neonates had antibody to HIV proteins that was not present in his/her own mother's serum.

IV. Examination of Antibody Responses in Serial Blood Samples

A. Prospective Study Group

In order to identify relationships between the presence (or absence) of antibody and clinical outcome, serial serum samples from 22 of the infants in the PSG were

tested side by side using immunoblots. Samples were obtained at least once prior to and at least once after 5 months of age. Two samples were collected from each of 7 infants, three samples from each of 9 infants, four from each of 4 infants, and five from 2 infants. The longest period of follow-up was 22 months. Table 1 shows the specific antibodies present in each sample and their relative intensities (judged visually) on immunoblot. The serologic results can be divided into four categories:

1. New antibodies appeared, as evidenced by either a new band or a more intense band. (n=1)
2. Antibodies present at birth disappeared and no new antibody appeared. (n=16)
3. Antibodies present at birth faded but were still present. (n=4)
4. Antibodies present at birth were still present and did not decrease in intensity. (n=1)

The clinical status of children belonging to each category of antibody reactivities is summarized in Table 2.

Figure 3 shows the immunoblots of two of the three serial samples from the one child (PSG #1) who developed new antibody. Antibody to gp41 and to p70, not present at 4 days of age, appeared by 5 months. In addition, antibody to p24 increased in amount. Passively acquired antibody to two of several EBV nuclear antigens (EBNA 1 and 2) completely disappeared by 5 months. Serum tested at 10 months of age (blot not shown) contained the same antibodies that were present at 5 months. The presence of new antibody is thought to be indicative of true infection. When last examined at 11 months the child was below the 5th percentile for height and weight and had delayed acquisition of motor skills (e.g. she did not bear weight at 9 months of age). She was classified as thriving poorly.

Sixteen of the children (PSG #2-16) who were seropositive at birth lost their antibody completely. One child developed AIDS (he was diagnosed as having PCP at 3 months and died at 9 months), six were thriving poorly, and nine were asymptomatic. The earliest that antibody disappeared was between 0 and 3 mos (PSG #3). The longest that antibody persisted before disappearing was 9 -13 mos (PSG#15). If age at loss of antibody is defined as halfway between the last positive sample and the first negative sample, the median age at which antibody disappeared was 5 months. In 5 of 7 symptomatic children who lost antibody, the loss occurred at or earlier than the median age of disappearance.

Four children in the PSG, still Ab-positive at the end of the study period, completely lost antibody to some of the HIV proteins and had progressively decreasing reactivity to others. An example of this "fading" pattern is shown in Figure 4. One of these children (PSG #18) had AIDS and died from PCP at 8 months, one child (PSG #19) was thriving poorly at 8 months with recurrent diarrhea and thrush, and the other two (PSG #16 and 17) were asymptomatic.

Only one child (PSG #22) had a constant antibody pattern during the entire study period. His immunoblots are shown in Figure 5. His earliest serum, from 4 months of age, recognized only one polypeptide cluster (p60/61). At 6 months and again at 10 months his reactivity was the same. This child was hospitalized 5 times during his first 6 months of life for diarrhea, fever, and pneumococcal sepsis. He fulfilled the criteria for AIDS when he was diagnosed as having PCP at age 10 months.

B. Symptomatic Study Group

Serial serum samples from six of the SSG children (i.e., children identified following clinical evidence of symptomatic HIV infection) were examined by Western immunoblot in an an attempt to learn what happens to the antibody response as disease progesses. The antibodies present in each sample are shown in Table 3. One patient showed strong reactivity to 7 out of 9 polypeptide clusters, including p24, gp41 and gp 120 on all 5 occasions tested over a two year period following presentation with LIP. The other subjects, 2 of whom died, had reactivities to fewer HIV proteins (only 1, 2, or 3 bands on immunoblot). All 5 of them either lost or never had reactivity to p24 during the test period, The size of the SSG is too small to allow correlation of immunoblot patterns with clinical status.

V. Immunoblots with Bridging Antibodies

Previous work had shown that antibody in children with AIDS may be difficult to detect by immunoblot and that the use of bridging anti-immunoglobulins can increase sensitivity[19]. Six serum samples which had been either negative or very weakly reactive by standard immunoblotting were retested using rabbit anti-human light chain as a bridging antibody. The samples were from 4 PSG infants (two of whom had AIDS, one of whom was thriving poorly, and one of whom was asymptomatic) and from 2 SSG children. The addition of bridging antibody increased the intensity of the bands representing cellular proteins, but did not reveal new reactivity to viral proteins as seen in Figure 6.

VI. Half-Life of Passively-Acquired Antibody

Densitometric reading of immunoblots was used to quantitate what was believed to be passively-acquired antibody against HIV polypeptides. Half-life was calculated as described in the Methods section for the antibodies to p17 and/or p60/61 in three PSG infants who had "fading antibody." These subjects were all asymptomatic and antigen-negative. The p17 and p60/61 antibodies were selected for the measurements because the intensities of these bands were in the range that could be accurately read by the densitometer. The results of the half-life determinations are shown in Table 4. The mean of the calculated half-lives was 38 ± 2.9 days.

VII. HIV Antigen Detection

Studies done in adults at risk for HIV infection suggest that viral antigen may circulate in serum prior to the appearance of antibody and that the reappearance of antigen in seroposite individuals may indicate progressive disease[77,80]. Therefore we wished to learn whether the presence of antigen in serum may help identify those children born to HIV Ab-positive mothers who are actually infected. The results of two p24 antigen detection assays are shown in Tables 5, 6, and 7, and summarized in Table 8. Fifty-six sera from 30 children were tested. Twenty of these were members of the PSG; 10 were SSG children. Eleven children had at least one serum sample tested by both assays, 10 had serum tested by the Abbott assay only, and 10 had serum tested by the DuPont assay only. Eighteen mothers

of these children were tested: 11 were mothers of PSG infants, 7 were mothers of SSG members.

Of the 48 individuals tested, 9 (19%) had at least one antigen-positive serum sample. The antigen positive individuals included 5 children and 4 mothers. One serum sample was Ag-positive by the Abbott assay but negative by the DuPont test. The remainder were either positive by both or only tested by one. (Of note, the "discrepant" sample had an antigen concentration by the DuPont assay which was 1.92 that of the negative control, very close to 2.00 cutoff for positivity.)

Two (10%) of the PSG infants tested had detectable antigen. The first was PSG infant #7. The antigen-positive sample from 8 months of age (the only serum tested) was antibody negative by immunoblot. This child was born at 34 weeks gestation with dysmorphic features (square head, flat face). When last examined at age 14 months she was small for her age (5th percentile) but otherwise asymptomatic. The other antigen-positive PSG infant, PSG #2, was described previously. He lost HIV antibody completely by 5 months and died of AIDS at 9 months. The appearance of antigen in this child coincided with the disappearance of antibody.

Three (30%) of the SSG children were antigen positive. The first was SSG #3, the older sibling of another Ag positive child with AIDS (PSG #2). This child died at 6 years from MAI infection. He was Ab positive by immunoblot; however, he only had antibody to one protein cluster (triplet 46/49/53) on all occasions tested. Another child in this family is also a member of the SSG (#5). He, however, was antigen negative. His clinical course has been much more indolent than his two siblings in that he has LIP but is doing well 2.5 years after presentation. The second SSG child who was Ag-positive, SSG #10, presented at 7 months of age

with meningococcemia, hepatosplenomegaly, and thrush. LIP was demonstrated by biopsy. This child developed a B cell CNS lymphoma and died of disseminated MAI at 31 mos. His only serum sample was antibody positive. Serum from the third SSG Ag-postive child (SSG #4) was positive by one assay and negative by the other. He was first tested for antibody at age 3 months and has maintained a constant pattern of weak reactivity to only two clusters of polypeptides (gp41 and p46/49/53). He had LIP and is growth retarded at 6 years of age.

Four of the 17 mothers tested were also antigen-positive. One is the mother of two antigen positive children with AIDS (PSG #2 and SSG # 3). Another is the mother of antigen-positive SSG #10 described above. The third is also a mother of a child in the SSG (SSG #6). This child has LIP and at age 8 years is doing well on very small doses of steroids. The fourth antigen-positive mother gave birth to twins, one of whom (SSG #8) has AIDS and has been antibody-positive. The other twin is antibody negative and healthy at age 7 years.

Of the 13 children (from either PSG or SSG) with AIDS who were tested for antigen 4 were antigen positive. None of the asymptomatic PSG children were antigen positive, and only one of the PSG subjects thriving poorly was antigen positive. Hence the test appears to be specific but not sensitive for symptomatic infection. Four of 8 mothers of children with AIDS had detectable antigen, while none of the 10 mothers of children who do not have AIDS were antigen-positive.

DISCUSSION

It was recently reported that one of every 61 infants born in New York City is HIV antibody positive[83]. This widely publicized finding has generated much concern and fear as it reflects the large number of women of child-bearing age infected with HIV. It is not known, however, how many of their infants are actually infected. Of the 25 infants followed prospectively in the present study, only three, or 12 percent, developed AIDS during the 10-25 month follow-up period. Although the study was not designed to determine the risk of vertical transmission of HIV, this finding suggests that either the risk of transmission is lower than the 33-65% estimates of previous studies[27,29,60], or that many infected children do not become ill until they are older than 1-2 years of age. Both are probably correct. Some early studies that determined transmission risk looked at infants born to women who already had one affected child and, as explained previously, they very likely over-estimated the risk of transmission. On the other hand, although several descriptions of pediatric AIDS state that most patients present prior to six months of age[28,31], there are several children in New Haven alone who did not present until 4-6 years of age, suggesting that longer follow-up of the 25 prospectively-identified subjects may reveal an infection rate higher than twelve percent. In addition, longer follow-up should clarify the clinical status of the 8 subjects who were "thriving poorly." These infants had signs of growth retardation, developmental delay and serious bacterial infections, consistent with, but not specific for, HIV infection. It is not clear to what extent these signs may be due to a poor social environment.

Immunoblotting was used in this study to detect antibody because it is the most specifc method available and because it allows identification of the individual HIV polypeptides recognized. Cell lines infected with two different HIV strains were used as antigen. This increased the ability to detect reactivity to two polypeptides (gp120 and p46/49/53) each of which was more prominent using one viral strain than the other. A negative control - antigen prepared from uninfected cells - was used to distinguish between reactivity to cellular and viral proteins. This control is not typically included in commercial Western blotting "kits." However, previous work in this laboratory, indicates that many uninfected individuals have circulating antibodies which react with proteins that are native to the cells used to grow HIV. These individuals would be identified as being HIV antibody-positive if their sera are not tested against this negative control.

The neonates of the PSG did not have antibodies to any polypeptides not recognized by their own mother's serum. Thus, as others have found, Western immunoblotting at birth is not usually useful in diagnosing congenital HIV infection.

Serial measurements of reactivity over time revealed several different patterns among the infants at risk. Appearance of new antibody, disappearance (either complete or partial) of antibody, and maintenance of antibody were all observed. Follow-up to date reveals no one pattern of antibody reactivity that stands out as a clear marker of infection. Nonetheless, the findings do raise some interesting possibilities.

Appearance of antibody to antigens not recognized at birth was seen in one subject by the age of 5 months. This finding, presumably indicative of infection, is important because it demonstrates the ability in at least some infected infants to

mount an antibody response against the virus. Although the antibody response in this case occurred in the absence of definitive clinical signs of HIV infection, it was accompanied by signs of poor health (growth retardation and developmental delay). It would thus appear that the presence of antibody does not necessarily portend a good outcome.

Most of the infants lost antibody during the study period. It is likely that this was maternal antibody acquired *in utero*. The length of persistence of the antibody ranged from 3 months to 13 months with a median of 5 months. Assuming exponential decay, the length of persistence of such antibody is dependent on the amount transferred from the mother to her fetus and on the half-life of the antibody. Densitometric quantitation of fading antibodies was performed for three subjects and was used to estimate roughly the half-life of maternal antibody to HIV. The calculated half-life of 38 days is somewhat longer than the 25-30 day half-lives of other passively acquired antibodies such as poliomeylitis antibody[84] and antinuclear antibody[85]. One potential problem with such a calculation is the possibility that the infants on whom it was based were, in fact, infected with HIV. In such a case disappearance of maternal antibody, as detected by immunoblot, might be hastened by the presence of circulating viral antigen. That is, antigen in the serum might compete with the antigen on the immunoblot for the antibody. To decrease the likelihood that this situation interfered with the determination of half-life, the antibody quantitations were conducted on asymptomatic, antigen-negative infants. It is also recognized that in order to accurately determine the antibody half-life a linear relationship between the concentration of a particular antibody in serum and the intensity of the corresponding band on immunoblot needs to be demonstrated. Knowing the half-life of maternal antibody could be quite useful in distinguishing

infected from non-infected infants. An infected infant might be expected to have his antibody disappear at either a faster or slower rate than predicted by the known half-life. Rapid fading might suggest high levels of circulating antigen, while slow fading might indicate production of antibody by the infant himself.

Interestingly, among all infants who lost antibody, most of those who were thriving poorly or had AIDS lost their antibody earlier than those who were asymptomatic. The difference was not statistically significant, perhaps because some of the infants "thriving poorly" were doing so for reasons unrelated to HIV infection. Since the length of persistence of maternal antibody depends on the amount present at the outset, these rapid losers of antibody may be infants who received less maternal antibody *in utero*. For other congenital infections it is believed that maternal antibody has a protective, viral neutralizing effect. If this is the case for HIV as well, the infants who received less of this antibody moiety transplacentally may have a higher rate of HIV infection or a more rapid onset of clinical disease.

Antibody, in general, whether passively acquired or actively produced, may have a protective role for the HIV-infected individual. This idea was proposed by other investigators in this laboratory when they found a diminished antibody response and more rapid onset of disease in children with AIDS compared to their parents[19]. In the present study, of all eight children with AIDS (from both the PSG and the SSG) only one had antibody to more than three polypeptides by the time clear signs of HIV infection were observed.

A diminished antibody response in infected individuals may allow the virus to exert its pathogenic effects or it may reflect increased viral replication and increased circulating antigen. Studies of adults have demonstrated the presence of antigen in

serum prior to the onset of clinical signs of HIV infection[80]. By analogy, we surmised that antigen testing might also be useful in diagnosing true HIV infection in infants. However, antigen detection assays performed in the present study did not support this. Four of five antigen positive children had AIDS. This suggests that detectable antigen is indicative of advanced HIV infection. The one antigen-positive child without AIDS has signs of poor health and probably is, in fact, infected. However, five other children with clear clinical evidence of HIV infection were antigen-negative. In addition, with only one exception, all infants without AIDS but at risk for infection were antigen-negative. These findings imply that antigen assays may not be sensitive for HIV infection, and that antigen is not detectable as an early marker of infection. Antigen testing of mothers, on the other hand, may be more useful. Although there were antigen-negative mothers who had infected children, all of the antigen-positive mothers had children who developed AIDS, suggesting that the presence of antigen in maternal serum may be a marker for infection in her child.

In conclusion, examination of antibody patterns and antigenemia in infants born to HIV-seropositive mothers did not yield any absolute markers of HIV infection. However, the results do suggest the following: 1) Many infected infants are capable of producing antbody against HIV proteins. 2) During the first two years of life, production of new antibody, disappearance of antibody present at birth, and maintainence of antibody present at birth are all associated with HIV infection. 3) Complete loss of antibody by 5 months of age may be suggestive of active infection. 4) A rough estimate reveals that the half-life of passively acquired maternal antibody is 38 days. 5) The presence of HIV core antigen (p24) in serum

is not a sensitive measure of HIV infection in infants, but does appear to be specific for symptomatic infection. 6) The presence of antigen in maternal serum may be a marker for infection in her offspring.

Table 1. HIV Antibodies in Prospective Study Group Serum Samples as Detected by Immunoblot

PSG Subject	Date of birth	Sample Date	HIV Antibodies 120	96	70	60/61	46/53	41	33/35	24	17	Clinical Status
1	10/31/86	11/86	—	—	+	++	++	++	+	—	—	THRIVING POORLY
		3/87	—	—	++	+++	++	++	+	++	—	< 5th %tile wt, ht
		7/87	—	—	++	+++	++	++	+	++	—	developmental delay
2	7/20/86	7/86	—	+	+	+	+	+	+	+	+	AIDS, died 5/87
		12/86	—	—	—	—	—	—	—	—	—	
		4/87	—	—	—	—	—	—	—	—	—	
3	2/17/87	2/87	++	—	—	++	—	—	+	+	+	THRIVING POORLY
		5/87	—	—	—	—	—	—	—	—	—	Neisseria meningitis age 3 wks.
												<5th %tile wt, ht
4	12/29/86	12/86	+	+	+	+	++	—	++	—	+	THRIVING POORLY
		7/87	—	—	—	—	—	—	—	—	—	<5th percentile wt, ht
		1/88	—	—	—	—	—	—	—	—	—	chronic active hepatitis

Key:

— No antibody apparent.
\+ Weak reactivity.
++ Moderate reactivity.
+++ Strong reactivity.

The +, ++, or +++ indicates the relative strength of reactivity as judged visually by intensity of the band. This reflects comparisons made between the serum samples of a single subject, not comparisons between one subject and another.

Table 1. continued

PSG Subject	Date of birth	Sample Date	HIV Antibodies 120	96	70	60/61	46/53	41	33/35	24	17	Clinical Status
5	3/18/86	6/86	+	—	—	—	+	—	+	+	+	THRIVING POORLY
		10/86	—	—	—	—	—	—	—	—	—	<5th percentile wt, ht
		1/87	—	—	—	—	—	—	—	—	—	
		5/87	—	—	—	—	—	—	—	—	—	
		1/88	—	—	—	—	—	—	—	—	—	
6	12/4/85	2/86	++	+	++	++	+++	+++	+++	++	++	THRIVING POORLY
		/86	—	—	+	++	++	++	++	++	++	falling off growth curve
		8/86	—	—	—	—	—	+	+	—	—	
		12/86	—	—	—	—	—	—	—	—	—	
		6/87	—	—	—	—	—	—	—	—	—	
7	9/1/86	9/86	—	++	+	++	+++	—	++	—	+	THRIVING POORLY
		5/87	—	—	—	—	—	—	—	—	—	<5th %tile ht, wt
		11/87	—	—	—	—	—	—	—	—	—	dysmorphic face
8	11/28/86	12/86	—	+	++	++	++	—	++	++	++	THRIVING POORLY
		1/87	—	+	—	+	++	—	++	++	++	<5th %tile wt
		10/87	—	—	—	—	—	—	—	—	—	lymphadenopathy
		1/88	—	—	—	—	—	—	—	—	—	

Table 1. continued

PSG Subject	Date of birth	Sample Date	HIV Antibodies 120	96	70	60/61	46/53	41	33/35	24	17	Clinical Status
9	11/28/86	12/86	—	+	++	++	++	—	++	++	++	ASYMPTOMATIC
		1/87	—	+	—	+	++	—	++	++	++	
		10/87	—	—	—	—	—	—	—	—	—	
		1/88	—	—	—	—	—	—	—	—	—	
10	6/14/86	6/86	—	+	+	+	+	+	—	+	—	ASYMPTOMATIC
		4/87	—	—	—	—	—	—	—	—	—	
11	3/30/86	4/86	—	—	+	—	—	—	++	—	—	ASYMPTOMATIC
		10/86	—	—	—	—	—	—	—	—	—	
12	9/4/86	9/86	++	+	+	++	+++	++	—	+++	++	ASYMPTOMATIC
		4/87	—	—	—	—	—	—	—	—	—	
13	11/28/86	1/87	++	+	++	++	—	—	—	—	—	ASYMPTOMATIC
		6/87	—	—	—	—	—	—	—	—	—	
14	11/5/86	2/87	—	—	—	+	—	—	—	—	—	ASYMPTOMATIC
		4/87	—	—	—	+	—	—	—	—	—	
		7/87	—	—	—	—	—	—	—	—	—	
		10/87	—	—	—	—	—	—	—	—	—	

Table 1. continued

PSG Subject	Date of birth	Sample Date	HIV Antibodies 120	96	70	60/61	46/53	41	33/35	24	17	Clinical Status
15	6/27/86	7/86	+++	+++	+	+++	+++	+++	+++	+++	+++	ASYMPTOMATIC
		12/86	—	—	—	++	++	++	+	+++	++	
		3/87	—	—	—	+	++	+	—	+	++	
		7/87	—	—	—	—	—	—	—	—	—	
16	3/9/86	4/86	—	+	++	+++	—	+	+	++	—	ASYMPTOMATIC
		1/87	—	—	—	—	—	—	—	—	—	
17	1/18/87	1/87	—	—	—	+	—	—	—	+	—	ASYMPTOMATIC
		5/87	—	—	—	—	—	—	—	—	—	
		1/88	—	—	—	—	—	—	—	—	—	
18	2/1/87	2/87	++	—	—	++	+	—	++	+	—	AIDS
		8/87	+	—	—	—	+	—	+	+	—	died 10/87
		10/87	—	—	—	—	+	—	—	—	—	?PCP
19	1/26/87	4/87	+	++	+	++	+++	+	++	++	+	THRIVING POORLY
		8/87	—	—	—	—	++	—	—	—	—	Diarrhea,thrush

Table 1. continued

PSG Subject	Date of birth	Sample Date	HIV Antibodies 120	96	70	60/61	46/53	41	33/35	24	17	Clinical Status
20	9/4/86	9/86	+++	+++	+++	+++	+++	+++	+++	+++	+++	ASYMPTOMATIC
		12/86	++	++	+++	+++	—	+++	+++	++	+	
		2/87	—	+	+	+	—	++	++	+	—	
21	8/19/86	8/86	+++	+++	+++	+++	+++	+++	+++	+++	+++	ASYMPTOMATIC
		10/86	++	+++	+++	+++	+++	+++	+++	+++	+++	
		3/87	—	—	++	++	+	+	—	+++	—	
22	12/29/86	4/87	—	—	—	—	—	+	—	—	—	AIDS
		6/87	—	—	—	—	—	+	—	—	—	fever, diarrhea,
		10/87	—	—	—	—	—	+	—	—	—	pneumococcal sepsis, PCP

Table 2. Clinical Status of PSG Children and Serial Antibody Reactivity Pattern

Antibody Reactivity Pattern	Number of Children			Total
	Asymptomatic	Thriving Poorly	AIDS	
1. New Antibody Appeared	0	1	0	1
2. Antibody Disappeared	9	6	1	16
3. Antibody Fading But Present	2	1	1	4
4. Antibody Unchanged	0	0	1	1

Table 3. HIV Antibodies in Symptomatic Study Group Serum Samples as Detected by Immunoblot

SSG Subject	Date of birth	Sample Date	HIV Antibodies 120	96	70	60/61	46/53	41	33/35	24	17	Clinical Status
1	9/30/83	1/85	—	++	—	++	++	++	—	++	—	AIDS
		10/85	—	++	—	++	++	++	—	+++	+	LIP
		9/86	—	++	—	++	++	++	—	+++	++	
		12/86	—	+++	—	++	+++	++	—	+++	++	
		4/87	—	+++	—	++	+++	++	—	+++	++	
2	1/15/85	5/86	—	—	—	—	—	++	—	+	—	AIDS, died age 2 years
		7/86	—	—	—	—	—	++	—	—	—	
		11/86	—	—	—	—	—	++	—	—	—	
3	6/16/80	8/15/85	—	—	—	—	—	++	—	—	—	AIDS
		6/30/86	—	—	—	—	—	++	—	—	—	MAI infection, died age 6 yrs.
4	1/8/82	8/16/84	+	—	—	—	+++	—	—	—	—	AIDS
		73/5/87	—	—	—	—	+++	—	—	—	—	LIP

Key:

— No antibody apparent.
\+ Weak reactivity.
++ Moderate reactivity.
+++ Strong reactivity.

The +, ++, or +++ indicates the relative strength of reactivity as judged visually by intensity of the band. This reflects comparisons made between the serum samples of a single subject, not comparisons between one subject and another.

Table 3. Continued

SSG Subject	Date of birth	Sample Date	HIV Antibodies 120	96	70	60/61	46/53	41	33/35	24	17	Clinical Status
5	8/12/83	8/85	+	—	—	—	+++	—	—	++	—	AIDS
		12/86	+	—	—	—	+++	—	—	++	—	LIP
		4/87	—	—	—	—	+++	—	—	—	—	
6	1/22/79	6/84	++	—	++	—	++	—	—	—	—	AIDS
		10/86	++	—	++	—	++	—	—	—	—	LIP

Table 4 Determination of Antibody Half -Life

PSG Subject*	HIV Antibody	Log[Ab]** in Serum Sample 1	Log[Ab] **in Serum Sample 2	Number of Days Between Samples	$t_{1/2}$***
20	p17 p60/61	2.46 2.84	2.01 1.67	51 144	34 37
21	p60/61	2.21	1.89	43	40
15	p17	2.94	2.23	95	40

Mean $t_{1/2}$ = 37.8 days
Standard deviation = 2.9 days

* Subject numbers correspond to those in Table 3.

** Antibody (Ab) quantity was determined by densitometry of immunoblot bands.

*** $t_{1/2}$ (half life of antibody in days) = $\dfrac{\log_{10}2}{\dfrac{\Delta\log[Ab]}{\text{time}}}$

Table 5 Results of Antigen Testing by Abbott and DuPont Assays for Prospective Study Group

PROSPECTIVE STUDY GROUP			
PSG Subject*	Sample Date	Abbott Assay	DuPont Assay
1	11/86	—	—
2	7/86 12/86 4/87	— + NT	— + —
3	4/87	NT	—
4	12/86	—	—
5	6/86 9/86 10/86 1/87	— — — —	NT NT NT —
6	2/86 6/86 8/86 12/86	— — — —	NT NT NT —
7	5/87	NT	+
12	4/87	NT	—
13	6/87	NT	—
14	2/87 4/87	— —	NT —
15	3/87	NT	—

PROSPECTIVE STUDY GROUP			
PSG Subject	Sample Date	Abbott Assay	DuPont Assay
19	4/87	NT	—
20	9/86 2/87	— —	NT —
21	8/86 10/86 3/87	— — NT	NT NT —
22	6/87	NT	—
23	6/87	NT	—
24	3/87	NT	—
25	11/86 2/87	— —	NT —

Key:
+ positive
— negative
NT not tested

* Subject numbers correspond to subjects in Table 1. Prospective Study Group (PSG) subjects 8, 9, 10, 11, 16,17 and 18 were not tested for antigen. PSG subjects 23, 24 and 25 did not have serial antibody testing and are not included in Table 1.

Table 6 Results of Antigen Testing by Abbott and DuPont Assays for Symptomatic Study Group

SYMPTOMATIC STUDY GROUP			
SSG Subject*	Sample Date	Abbott Assay	DuPont Assay
1	2/85	—	NT
	12/86	NT	—
2	5/86	—	NT
	7/86	—	NT
	11/86	—	NT
3	8/85	+	NT
	6/86	+	NT
4	8/84	+	NT
	3/87	+	—
5	8/85	—	NT
	12/86	—	NT
6	10/80	—	NT
	11/82	—	NT
	6/84	—	NT
	1/87	—	NT
7	5/87	NT	—
8	4/87	—	—
9	2/85	—	NT
10	1/85	+	NT

Key:
+ positive
— negative
NT not tested

* Subject numbers correspond to subjects in Table 3. Symptomatic Study Group (SSG) subjects 7-10 did not have serial antibody testing and are not included in Table 3.

Table 7. Results of Antigen Testing by Abbott and DuPont Assays for Mothers of Prospective and Symptomatic Study Groups

MOTHERS OF CHILDREN IN PROSPECTIVE STUDY GROUP		
Mother of PSG Subject*	Abbott Assay	DuPont Assay
1	—	—
2	+	+
6	—	—
13	NT	—
14	—	—
15	—	—
20	—	—
21	—	NT
23	—	NT
24	NT	—
25	—	—

MOTHERS OF CHILDREN IN SYMPTOMATIC STUDY GROUP		
Mother of SSG Subject*	Abbott Assay	DuPont Assay
2	—	NT
3	+	+
4	—	NT
5	+	+
6	+	NT
8	+	+
9	—	—
10	+	NT

Key:
+ positive
— negative
NT not tested

* Subject numbers correspond to PSG and SSG subjects in Tables 3, 4 and 5.

Table 8. Results of p24 Antigen Assay in PSG and SSG Children

Study Group	Number of Children or Mothers with Positive Test/Number Tested: By Abbott Assay Only	By DuPont Assay Only	By Both Abbott and DuPont Assays	Total (percent)
PSG Children	0/2	1/9	1/9	2/20 (10)
SSG Children	3/7	0/1	0/2	3/10 (30)
PSG Mothers	0/1	0/3	1/7	1/11 (9.1)
SSG Mothers	2/5	0/0	1/1	3/6 (50)

FIGURE 1. HIV ANTIBODY DETECTION ON A WESTERN IMMUNOBLOT. Serum from a neonate born to an HIV-antibody postive mother was reacted with an immunoblot containing extracts from 4 different cell lines: X50-7.8 (derived from EBV-transformed B cells), X50-7.8/PH1-MN (X50-7.8 cells infected with HIV strain PH1-MN), H9 (derived from a T cell leukemia line), and H9/RF2 (H9 cells infected with HIV strain RF2). The EBV nuclear antigens, EBNA 1 and EBNA 2, are present in the X50-7.8 and X50-7.8/PH1-MN extracts. Note multiple reactivities to cell-associated proteins in the uninfected cell lines (X50-7.8 and H9) and a wide spectrum of reactivities to a full array of viral polypeptides in the infected cell lines (X50-7.8/PH1-MN and H9/RF2). Numbers on the left are molecular weight markers.

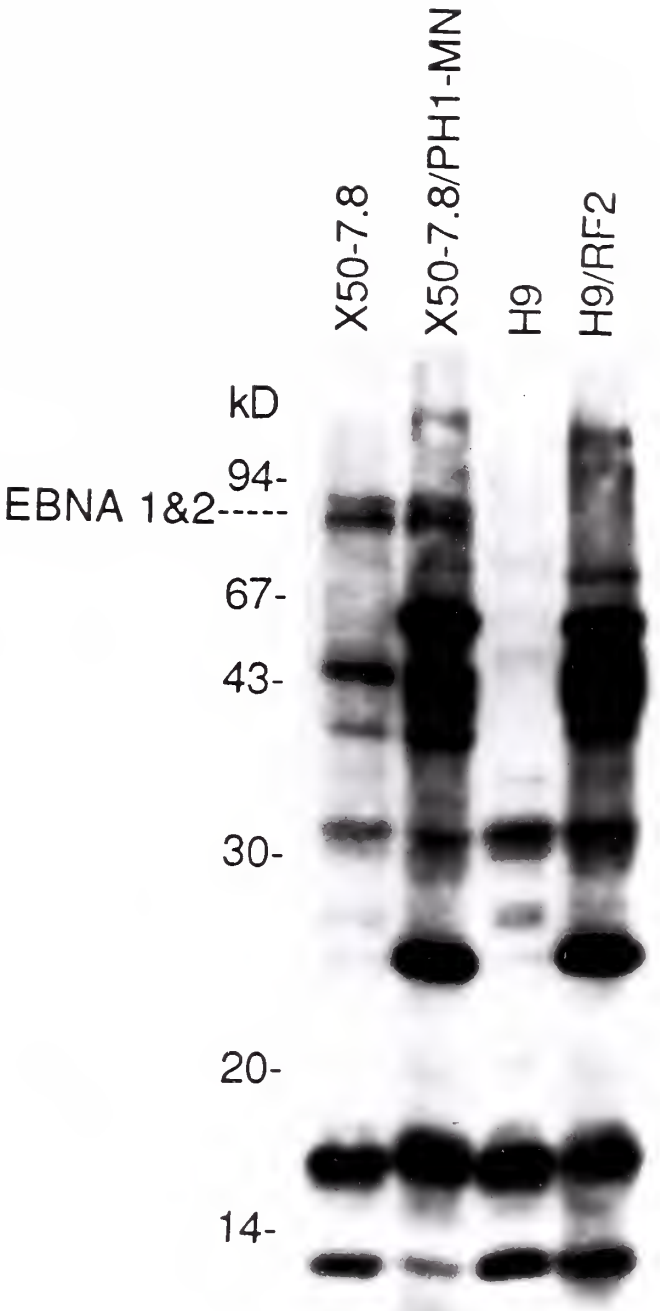

X50-7.8
X50-7.8/PH1-MN
H9
H9/RF2
kD
94-
EBNA 1&2-----
67-
43-
30-
20-
14-

FIGURE 2. COMPARISON OF HIV ANTIBODIES IN SERUM OF AN INFANT AND HIS INFECTED MOTHER.

The particular HIV antibodies (as well as their relative quantities) present in the serum of the neonate appear to be the same as those present in the serum of his mother.

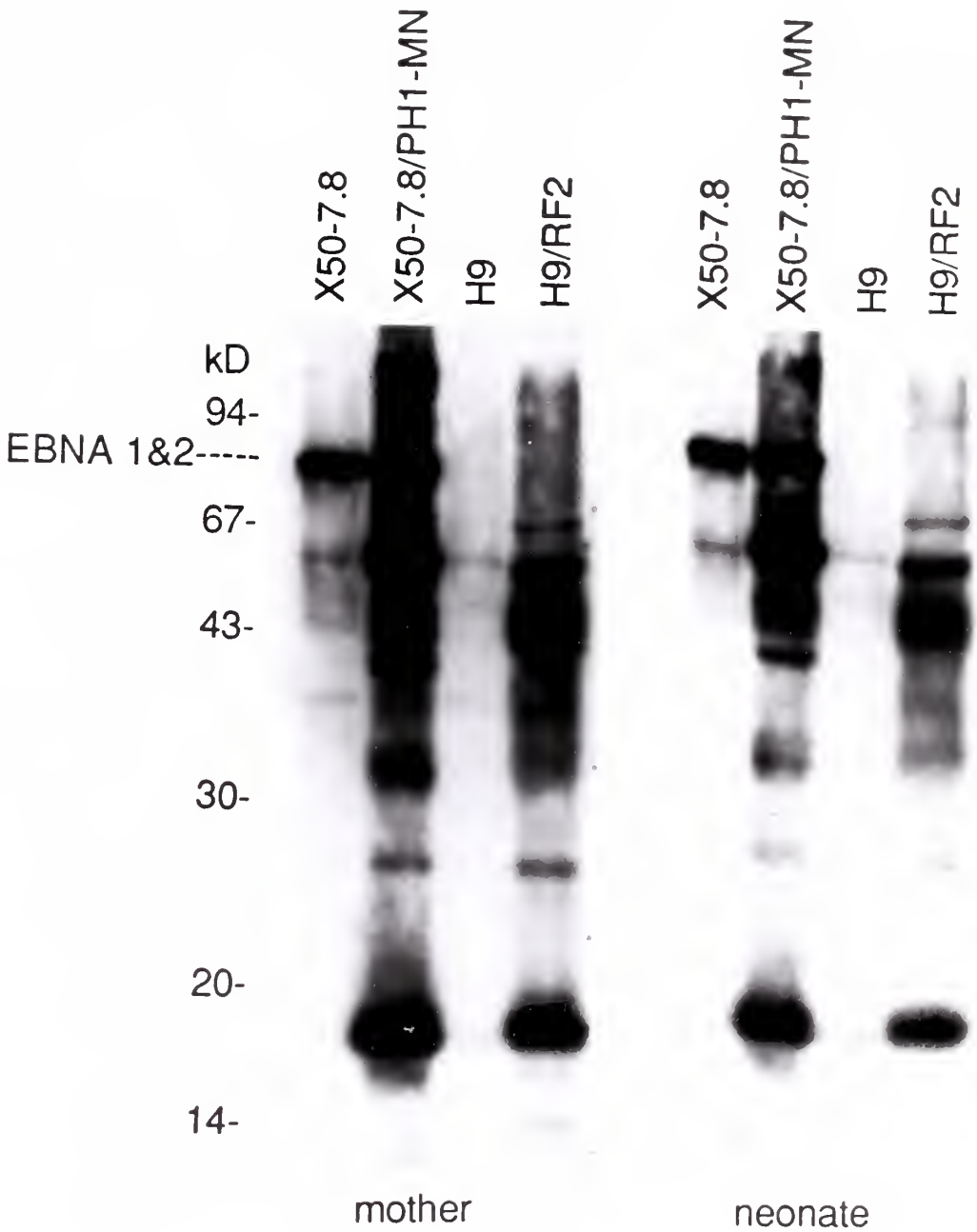

X50-7.8
X50-7.8/PH1-MN
H9
H9/RF2
X50-7.8
X50-7.8/PH1-MN
H9
H9/RF2
kD
94-
EBNA 1&2-----
67-
43-
30-
20-
14-
mother
neonate

FIGURE 3. APPEARANCE OF NEW ANTIBODIES TO HIV IN AN INFANT BORN TO AN HIV ANTIBODY-POSITIVE MOTHER.

During the first 5 months of life there has been marked intensification of the antibody response to p24 and acquisition of antibody to gp41 and p70. There has been concurrent loss of antibody to the EBV nuclear antigens.

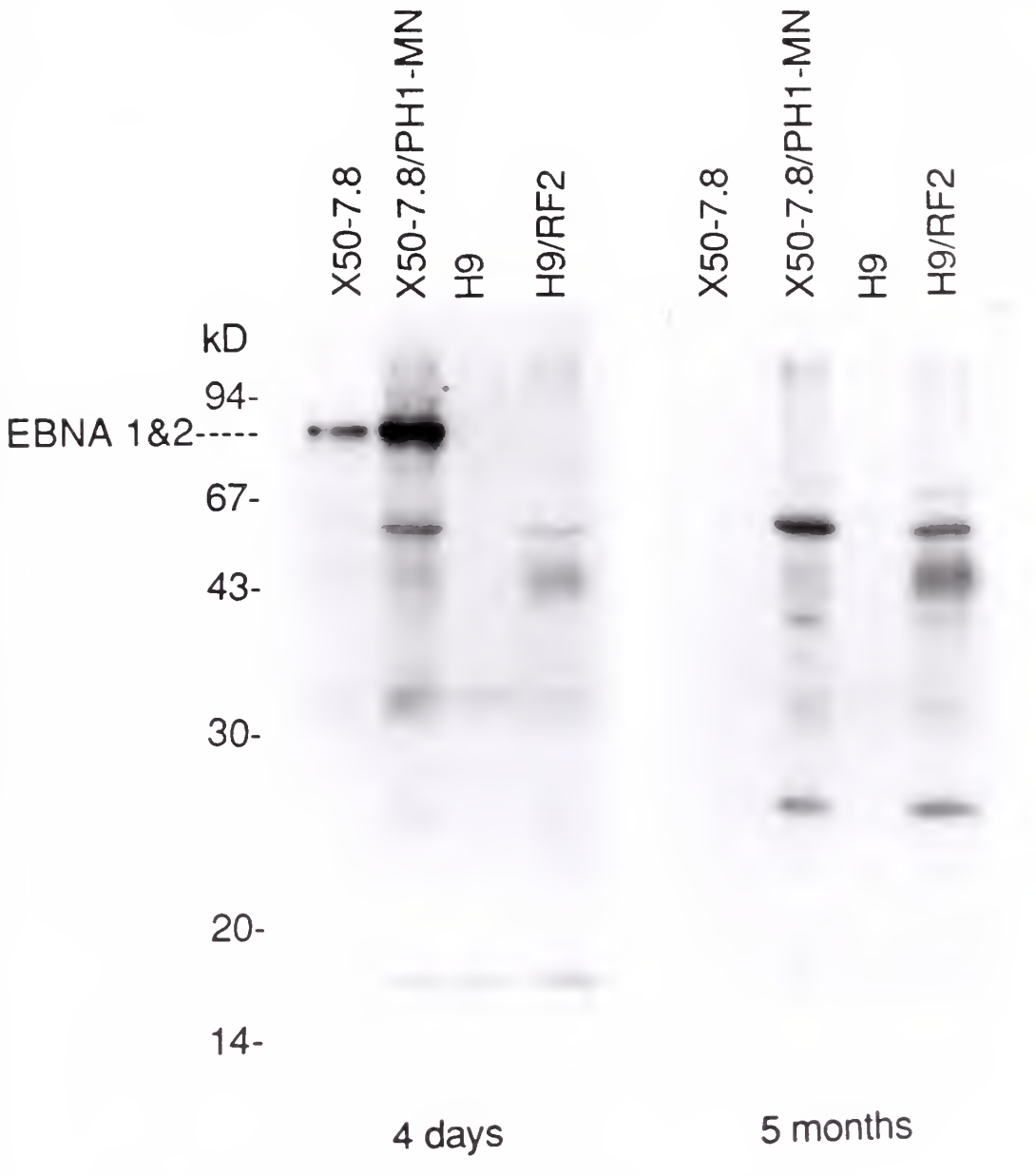

X50-7.8
X50-7.8/PH1-MN
H9
H9/RF2
X50-7.8
X50-7.8/PH1-MN
H9
H9/RF2
kD
94-
EBNA 1&2-----
67-
43-
30-
20-
14-
4 days
5 months

FIGURE 4. PROGRESSIVE LOSS OF ANTIBODIES TO HIV IN AN INFANT BORN TO AN HIV ANTIBODY-POSITIVE MOTHER.

Over the first 6 months of life reactivities to the 9 HIV polypeptide clusters have diminished or become undetectable.

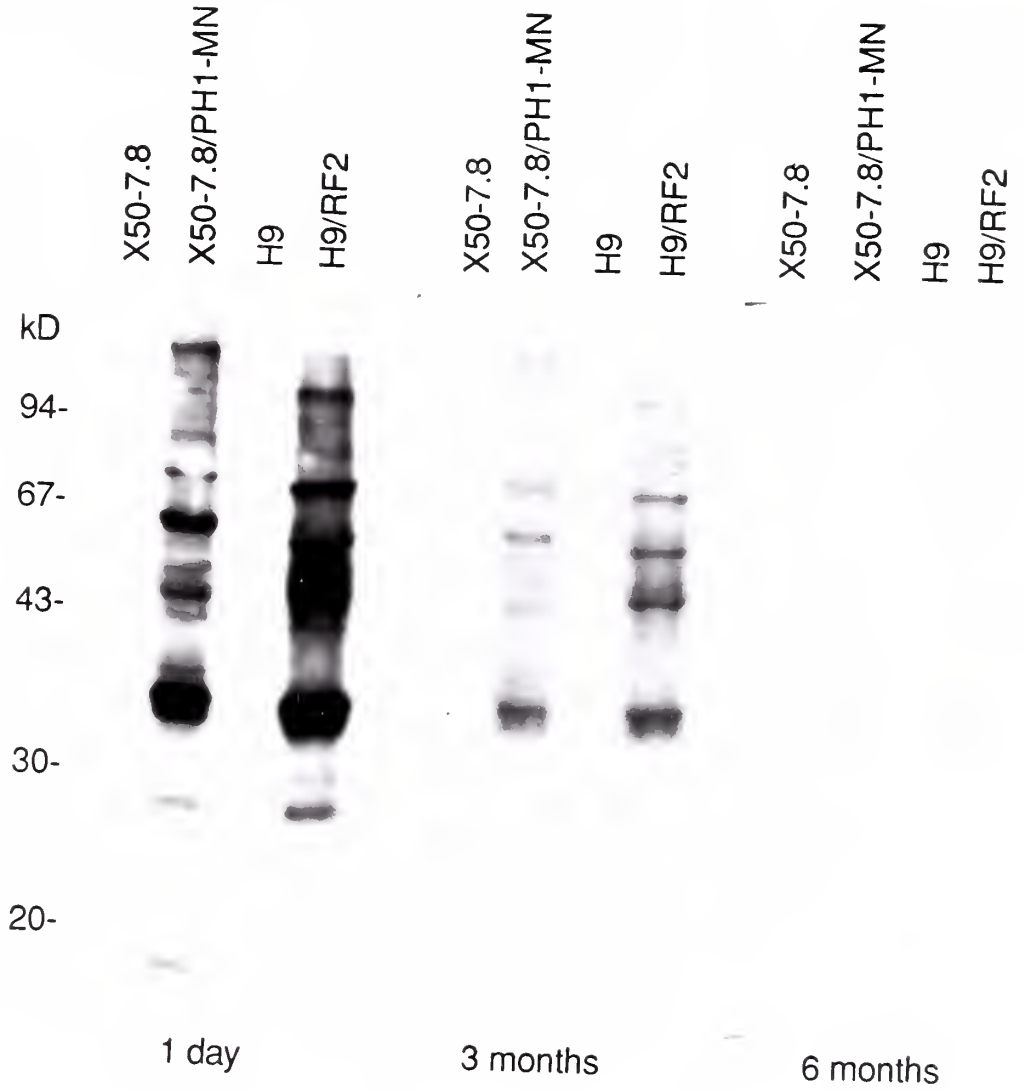

X50-7.8
X50-7.8/PH1-MN
H9
H9/RF2
X50-7.8
X50-7.8/PH1-MN
H9
H9/RF2
X50-7.8
X50-7.8/PH1-MN
H9
H9/RF2
kD
94-
67-
43-
30-
20-
1 day
3 months
6 months

FIGURE 5. CONSTANT PATTERN OF HIV ANTIBODY IN AN INFANT BORN TO AN HIV ANTIBODY-POSITIVE MOTHER.

This infant with full-blown AIDS has reactivity to only one cluster of HIV polypeptides (p60/61) on all three occasions tested in the first 10 months of life.

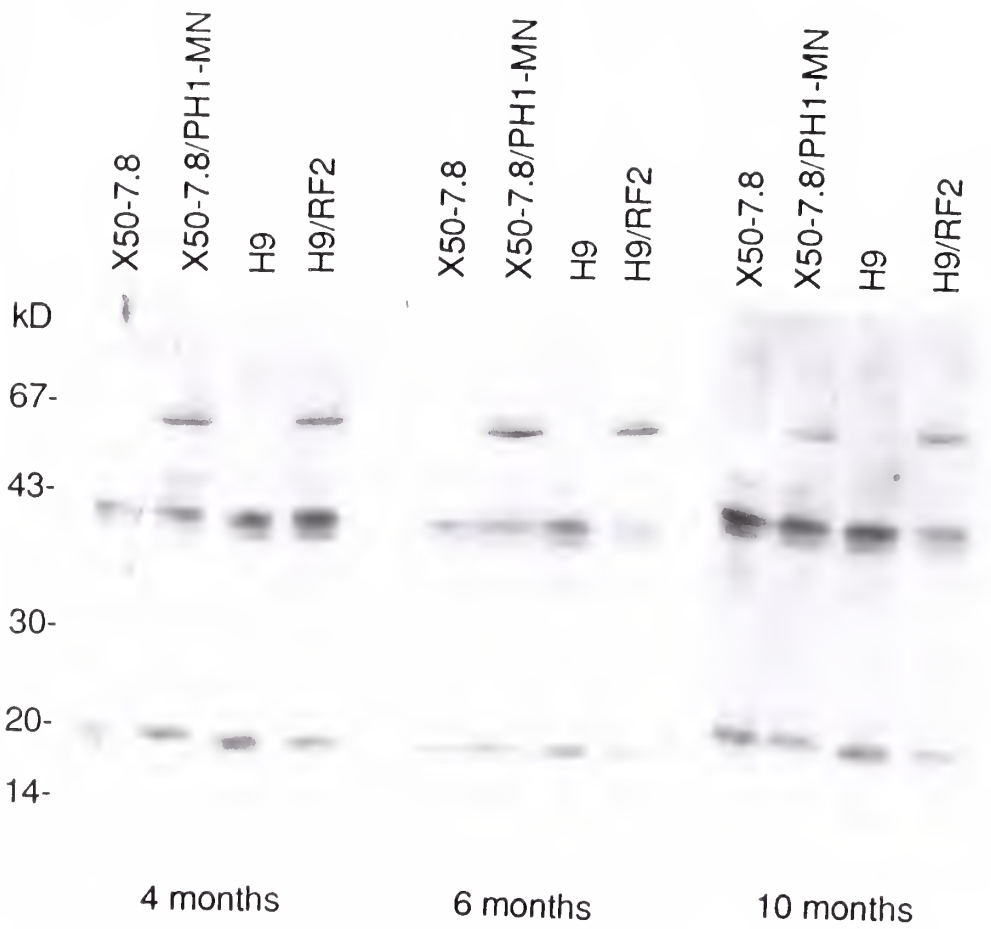
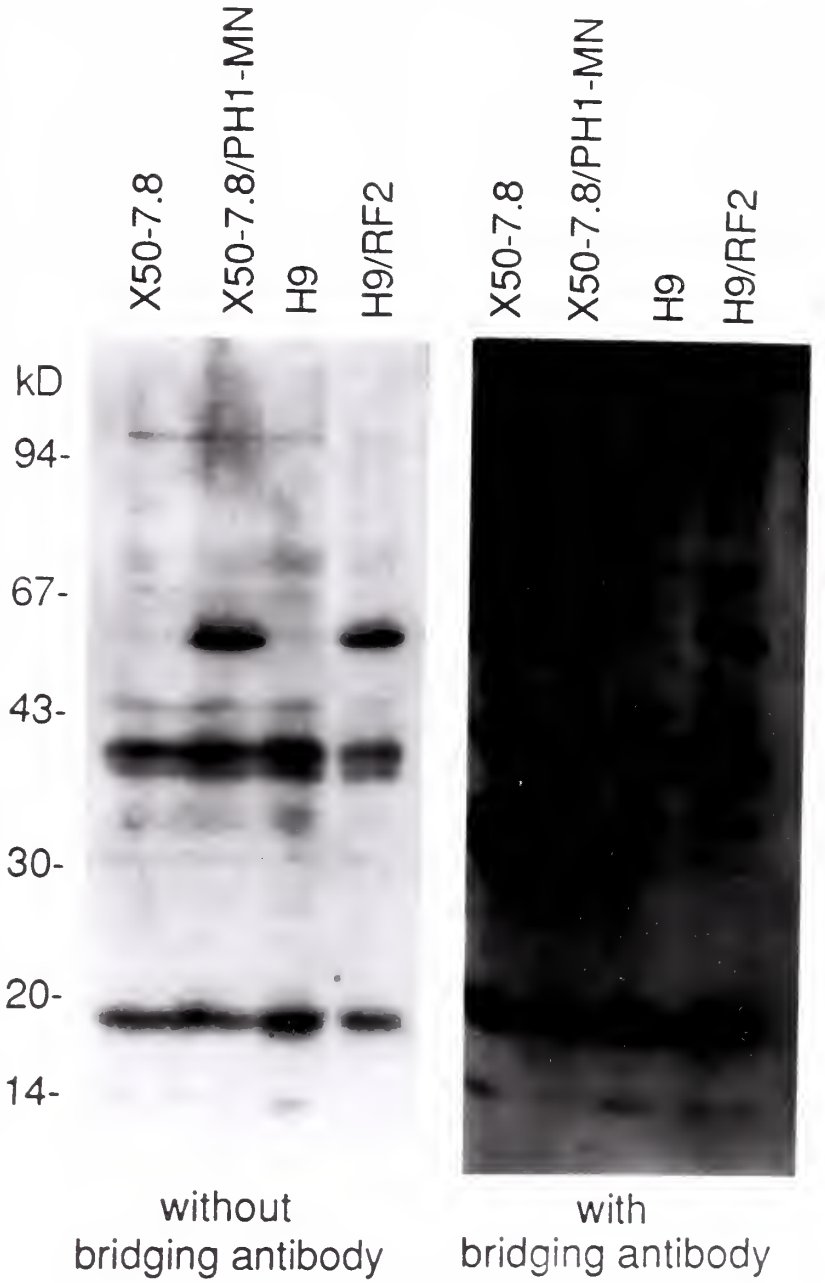

X50-7.8
X50-7.8/PH1-MN
H9
H9/RF2
X50-7.8
X50-7.8/PH1-MN
H9
H9/RF2
X50-7.8
X50-7.8/PH1-MN
H9
H9/RF2
kD
67-
43-
30-
20-
14-
4 months
6 months
10 months

FIGURE 6. THE USE OF BRIDGING ANTIBODY WITH WEAKLY REACTIVE SERUM.

The addition of rabbit anti-human light chain immunoglobulin following incubation of the immunoblot with serum does not reveal reactivities to any HIV polypeptides which were not detected without bridging antibody. The bands at 30-35 kDa in the X50-7.8 and X50-7.8/PH1-MN lanes of the bridging Ab immunoblot reflect antigens in the X50-7.8 cells recognized by anti-human light chain immunoglobulin.


X50-7.8
X50-7.8/PH1-MN
H9
H9/RF2
X50-7.8
X50-7.8/PH1-MN
H9
H9/RF2
kD
94-
67-
43-
30-
20-
14-
without
bridging antibody
with
bridging antibody

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